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Comparison of the occurrence of fungi causing postharvest diseases of apples grown in organic and integrated production systems in orchards in the Czech Republic

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During the years 2013–2015, the occurrence of fungi causing postharvest diseases of apples was evaluated in three apple orchards with integrated and organic production systems in the Czech Republic. The postharvest disease fungi were more frequently recorded in fruits from organically cultivated apple trees (average 29.97%) than those from integrated production (17.67%). This difference is statistically significant.

Neofabraea, *Penicillium*, *Alternaria* and a sterile grey mycelium were the most frequently recorded taxa in apples grown in organic and integrated production systems. There were also significant differences in the frequency of occurrence of the most frequent fungal taxa between years. In 2013 and 2014, *Neofabraea* was the most frequently isolated genus in both cultivation systems, whereas in 2015, *Penicillium* was the most frequently recorded genus also in both cultivation systems, which was likely caused by the low precipitation in the 2015 growing season.

Key words: *Malus domestica*, organic production, integrated production, *Neofabraea*, *Penicillium*.

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Novotný D., Lukáš J., Brožová J., Růžičková P. (2019): Srovnání výskytu hub způsobujících skládkové choroby jablek vypěstovaných v sadech s organickým a integrovaným systémem produkce v České republice. – Czech Mycol. 71(1): 99–121.

V letech 2013–2015 byl hodnocen výskyt hub způsobujících skládkové choroby jablek ve třech jabloňových sadech s integrovaným a ekologickým systémem produkce v České republice. Houby způsobující skládkové choroby byly častěji zaznamenány u plodů z jabloní pěstovaných v ekologické produkci (průměrně 29,97 %) než u integrované produkce (17,67 %). Rozdíl je statisticky významný.

Nejčastěji zaznamenávanými taxony v jablkách pěstovaných v ekologické a integrované produkci byly *Neofabraea*, *Penicillium*, *Alternaria* a sterilní šedé mycelium. Rovněž byly zjištěny rozdíly v četnosti výskytu nejčastěji se vyskytujících taxonů hub mezi jednotlivými lety. V letech 2013 a 2014 byl rod *Neofabraea* nejčastěji izolovaným identifikovaným taxonem v obou systémech pěstování, zatímco v roce 2015 byl v obou systémech nejčastěji zaznamenávaným taxonem rod *Penicillium*, což bylo pravděpodobně způsobeno nízkými srážkami ve vegetační sezóně 2015.

INTRODUCTION

The apple tree belongs to the most important fruit trees in temperate zones worldwide, including the Czech Republic. In the Czech Republic, 6,000 hectares have been planted with apple trees, from which more than 122,000 tonnes were harvested in 2017 (Buchtová 2018). Golden Delicious, Idared, Jonagold, Rubín, Bohemia, Gala, Šampion, Spartan, McIntosh and Topaz are the most frequently planted cultivars in the Czech Republic. The cultivars are grown in different fruit tree shapes; slender spindle, undershrub and bush tree shapes are most commonly used in commercial orchards (Buchtová 2018).

Apple trees are grown in conventional, integrated and organic production systems. The use of pesticides for disease management in organic apple orchards is limited. In this production system, the use of compost, suspended rock powder, copper and sulphur compounds, botanical and fungicidal soaps, traps and biological methods is allowed (Holb et al. 2015). All available methods and means (incl. synthetic pesticides) are allowed in the integrated production system to control harmful organisms as far as it is economically viable. The used methods and means have to reduce or minimise risks to human health and damage to the environment (Kocourek 2015).

Apple trees are hosts to many phytopathogenic fungal species which can cause pre- and also postharvest diseases. *Venturia inaequalis* and *Podosphaera leucotricha* are the most important preharvest fungal pathogens (Holb et al. 2015).

Apples are commonly stored for a long time (up to 4–12 months) using different procedures, e.g. storage at low temperature (around 4 °C) or under ULO (ultralow oxygen atmosphere). In spite of this, a considerable amount (up to 10–20%) of harvested apples may be lost during storage, resulting from the activity of microorganisms (mainly fungi) which cause postharvest diseases. Fungi from the genera *Penicillium*, *Botrytis*, *Colletotrichum*, *Monilinia*, *Mucor*, *Fusarium*, *Alternaria* and *Neofabraea* (formerly *Gloeosporium*) cause postharvest diseases most frequently. More than one species of each of these fungal genera can cause postharvest apple disease (Rosenberg 1990, Turechek 2004).

Many studies focused on the occurrence of fungi causing postharvest diseases of apples have been published from different parts of the world (e.g. Sholberg & Haag 1995, Valiuškaitė et al. 2006, Konstantinou et al. 2011, Ivić et al. 2012, Alwakeel 2013, Børve et al. 2013, Grantina-Ievina 2015, Juhnevica-Radenkova et al. 2016). Nevertheless, the number of studies comparing the occurrence of fungal species causing postharvest diseases of fruits harvested from organic and integrated apple orchards is very limited. Many of the studies are focused on a selected group of fungi causing postharvest diseases of apples (e.g. *Fusarium*, *Neofabraea*, *Penicillium*) (Sanderson & Spotts 1995, Jones et al. 1996, Amiri &

Bompeix 2005, Sever et al. 2012). Postharvest fungal diseases of stored apples grown in organic and integrated production systems were investigated by DeEll & Prange (1993), Blažek et al. (2006) and Jönsson et al. (2009). However, they classified the species according to the observed disease symptoms on fruits. Attention was also paid to the composition of mycobiota of only healthy stored apples grown in organic and integrated production systems (Granado et al. 2008) and endophytic mycobiota of apples before harvest (Camatti-Sartori et al. 2005).

The objective of our research was to compare the occurrence of fungi causing postharvest diseases of apples grown in orchards with an integrated production system to those with an organic system.

Material and methods

Study sites and cultivars. The differences between the occurrences of the most important fungi causing postharvest diseases of apples were evaluated on apples produced in three orchards with integrated or organic systems in the Czech Republic (Česká Skalice, Holovousy and Prague-Ruzyně) in the years 2013–2015.

We evaluated apple cultivars Florina, Goldstar, Rosana and Topaz, which are scab-resistant cultivars, and cultivars Angold, Bohemia, Golden Delicious, Idared, Melrose, Rubín and Šampion, which are scab-susceptible cultivars.

Locality Holovousy is situated in East Bohemia (close to the town of Hořice, Jičín District) at an altitude of 283–307 m, has a mean annual temperature of 9.5 °C, a mean annual precipitation of 598.6 mm (Figs. 1, 2), a slope of 1%, haplic luvisol soil and a loamy soil texture. Eight cultivars (Angold, Bohemia, Florina, Golden Delicious, Idared, Melrose, Rubín and Topaz) were evaluated in the integrated system and seven cultivars (Angold, Florina, Golden Delicious, Rosana, Rubín, Melrose and Topaz) in the organic system. All variants were slender spindle, undershrub or bush trees. In 2013–2015, at least 15 and 7 fungicides were used to protect apple trees in integrated and organic production systems, respectively, every year (Tab. 1).

Locality Prague-Ruzyně is situated in Central Bohemia on the western outskirts of the city of Prague near the airport, has an altitude of 349–358 m, a mean annual temperature of 9.7 °C, a mean annual precipitation of 545 mm (Figs. 1, 2), a slope of 6% and loamy soils classified as haplic luvisols. Six cultivars (Goldstar, Melrose, Rosana, Rubín, Šampion and Topaz) cultivated in both systems (organic, integrated) were monitored. All variants were slender spindle, undershrub or bush trees. In 2013–2015, every year at least 10 and 6 fungicides were used to protect apple trees in integrated and organic production systems, respectively (Tab. 2).

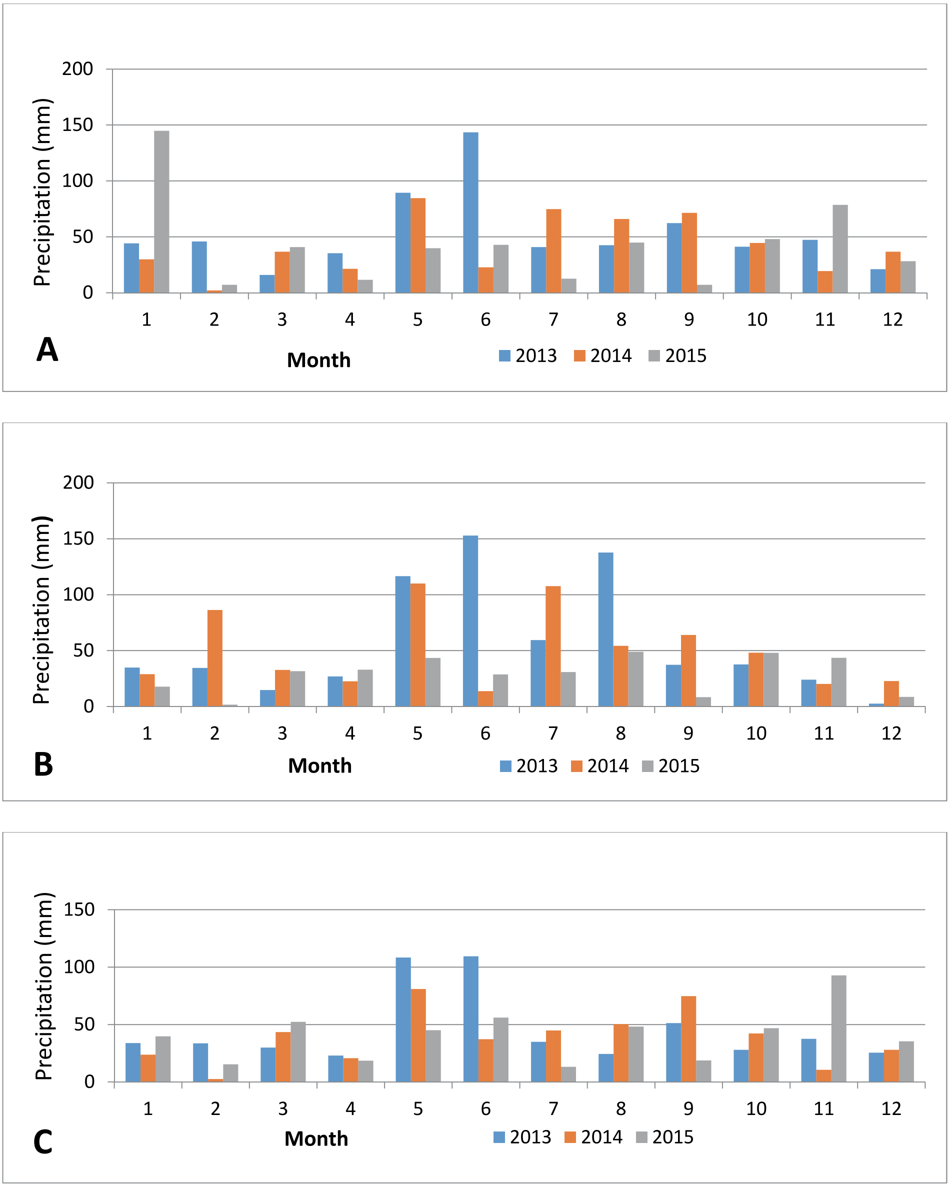

Fig. 1. Monthly precipitation at (A) Holovousy, (B) Prague-Ruzyně and (C) Česká Skalice in 2013–2015.

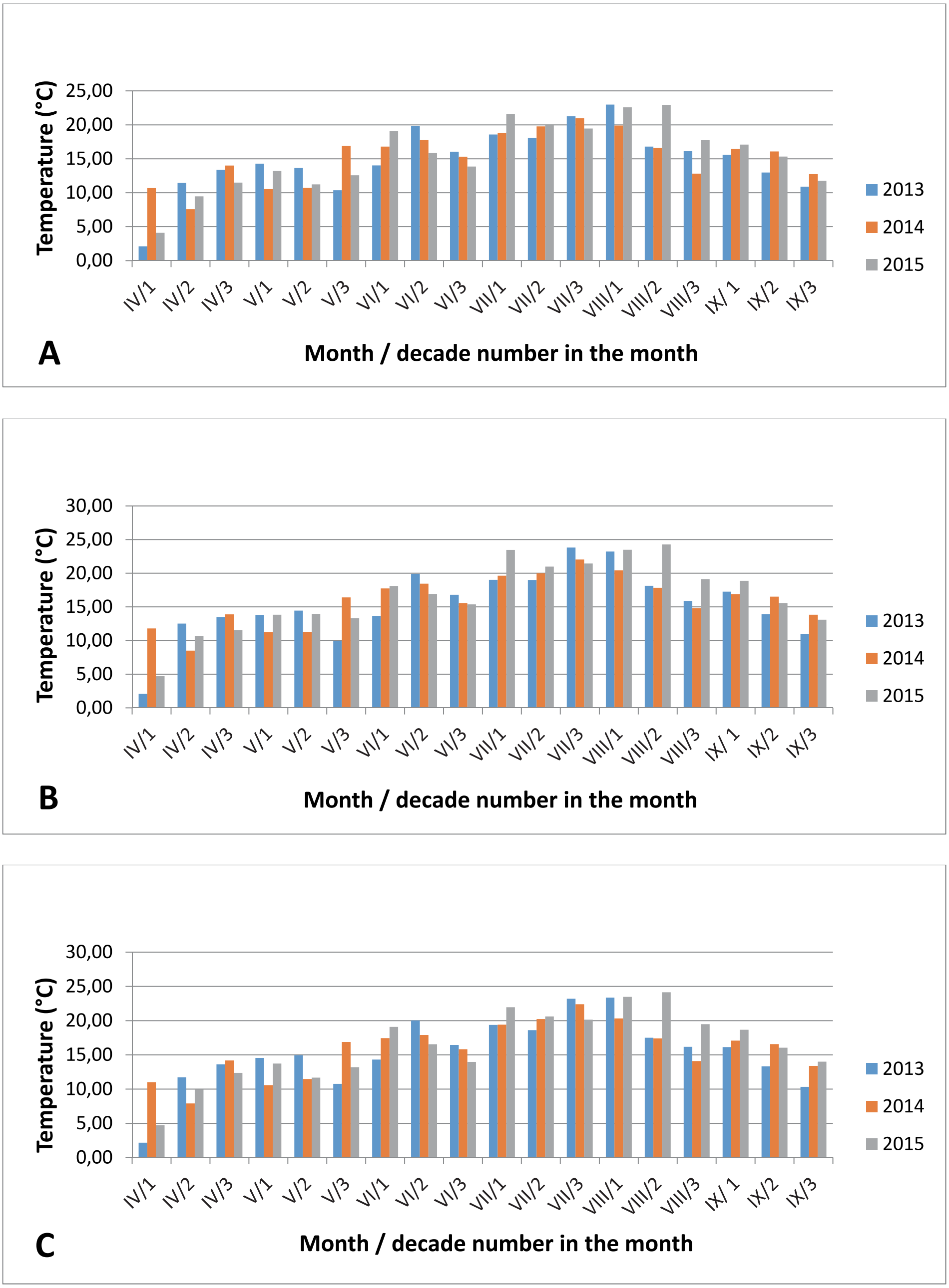

Fig. 2. Mean temperatures per decade at (A) Holovousy, (B) Prague-Ruzyně and (C) Česká Skalice from April to September 2013–2015.

Locality Česká Skalice is situated in East Bohemia (Náchod District), has an altitude of 289–346 m, a mean annual temperature of 9.5 °C, a mean annual precipitation of 588 mm (Figs. 1, 2), a slope of 3–4%, sand-loam soil. Apples of four cultivars (Bohemia, Goldstar, Šampion, Topaz) grown in the integrated production system were evaluated, all variants being slender spindle, undershrub or bush trees. Every year at least 14 fungicides were used to protect apple trees in the integrated production system in 2013–2015 (Tab. 3).

Tab. 1. Application of plant protection products (fungicides) in Holovousy orchard with integrated and organic production systems (number of fungicide applications).

Name of fungicide	2013		2014		2015	
	integrated	organic	integrated	organic	integrated	organic
Alginure		4		4		4
Antre 70 WG			1			
Captan 80 WG	3		3		2	
Clarinet 20 SC	1		1			
Delan 700 WDG	3		1		2	
Delan 700 WG			1			
Dithane DG			2		2	
Dithane Neo Tec	1					
Domark 10 EC					1	
Flint Plus	1					
Funguran-OH	1		1		1	
Champion 50 WP		2		2		2
Chorus 50 WG					2	
Chorus 75 WG	1		2		1	
Kumulus WG		4	1	4		4
Merpan 80 WG	1				1	
Mycosin		2		2		2
Mythos 30 S	1		2		1	
Polisenio		6		6		6
Polyram WG			1		1	
Score 250 EC	4		3		2	
Sulikol K		5		5		5
Sulikol 750 SC	1					
Syllit 400 SC	3		2		2	
Talent	1		3		1	
Thiram Gran.	2		2		2	
Topas 100 EC			1		1	
Zato 50 WG	1					
Vitisan		4		4		4

Tab. 2. Application of plant protection products (fungicides) in Prague-Ruzyně orchard with integrated and organic production systems (number of fungicide applications).

Name of fungicide	2013		2014		2015	
	integrated	organic	integrated	organic	integrated	organic
Alginure		5		4		2
Antre	1					
Captan					3	
Delan 700 WDG	1		3		1	
Dithane M 45	1					
Domark 10 EC			1		1	
Champion 50 WP				1		
Chorus 75 WG	1		2		1	
Kumulus		6	1	5	1	8
Merpan 80 WG	1		1		2	
Mycosin	2	1		5		4
Mythos			1		1	
Polisenio		3		1	1	2
Punch 10 EW	1		1		1	
Score 250 EC	1		2		2	
Sulfurus				4		1
Sulikol K	1	2				
Syllit 400 SC	1		2			
Talent	1		1		1	
Thiram Granuflo	3					
Vitisan		2		7		5

Tab. 3. Application of plant protection products (fungicides) in Česká Skalice orchard with integrated production system (number of fungicide applications).

Name of fungicide	2013	2014	2015
Antre 70 WG	1		1
Atos			1
Bellis			1
Captan 80 WG		2	1
Delan 700 WDG	3	2	2
Delan 700 WG			
Discus	1	1	1
Dithane DG Neotec	3	3	2
Domark 10 EC			1
Flint plus	2	1	1
Flowbrix	1	1	
Champion 50 WP			1

Name of fungicide	2013	2014	2015
Integro			1
Kumulus WG	2	2	1
Luna Experience			1
Merpan 80 WG	5	1	1
Mythos 30 S	3	2	1
Polyram WG	1	2	
Score 250 EC	2	1	
Syllit 400 SC	1	1	2
Talent			1
Tercel	1	1	
Thiram Granuflow	1	1	1

Evaluation of fungal colonisation. In the harvest time, one hundred healthy fruits (without visible damage) from each evaluated variant (combinations of cultivar, orchard site and production system) was picked and stored in cooling box at 5 °C. From early January the stored apples were checked every two weeks until the end of the recommended storage time (end of March to end of May). Visually infected fruits were discarded, their surface was sterilised with ethanol and 5 pieces (approx. 3–5 × 3–5 × 1–2 mm) of the damaged part (skin and flesh) of each fruit were incubated on 2% malt agar medium with chloramphenicol (100 mg/l) to identify the fungal taxa. The identification of fungi growing in vitro cultures was based on morphological features only. Most isolates were identified on genus level only, because without sequencing their DNA it is very difficult to classify the majority of fungi to species level. Sequencing of the DNA of all obtained strains would be very expensive.

The frequency of occurrence of the fungal taxa in each apple samples was evaluated. Identification of genera and species was performed using morphological characteristics. The following references were used for identification (based on macro- and micromorphology): Ellis (1971, 1976), Jones & Aldwinckle (1990), Verkley (1999), de Hoog et al. (2000), Samson et al. (2004), Domsch et al. (2007).

Statistical analysis. A recursive partitioning approach was used for non-parametric regression of fungus occurrence for the studied variables (production system, variety, locality, year, frequency of occurrence). All analyses were performed using R (R freeware statistical package; Anonymus on-line).

RESULTS

Overall assessment

During 2013–2015, 9,200 apples were picked in three orchards, and 2,007 fungal isolates were obtained. Fungi causing postharvest diseases were more frequently recorded in fruits from trees planted in organic orchards (on average 29.97% of apples) than fruits from trees in orchards with the integrated production system (17.67%). The difference was statistically significant (Figs. 3, 5, 6; Tabs. 4, 5).

Fungi causing postharvest diseases were most frequently recorded in 2013 and least frequently in 2015 in both production systems (Fig. 4). Differences in occurrence of the most frequently fungal taxa between 2015 and both previous years (2013, 2014) were found. In 2013 and 2014, the genus *Neofabraea* was the most frequently isolated fungal genus from apples grown in both cultivation systems, but in 2015 *Penicillium* was the most frequently isolated fungal genus in all orchards (Tabs. 5, 6, 7, 8). The diversity of fungi in both production systems was approximately the same. A total of 19 and 20 fungal taxonomic groups were isolated from apples grown in the organic and the integrated production system, respectively (Tab. 5).

In total, fungi of 21 taxonomic groups were found. *Neofabraea* (16.97% of apples), *Penicillium* (2.87%), sterile grey mycelium (2.16%), *Fusarium* (1.71%), *Alternaria* (1.65%) and a sterile light mycelium (1.39%) were the most frequently recorded taxa from apples grown in the organic production system (Tab. 5). The organically grown apples originating from the orchard at Holovousy were damaged by postharvest diseases caused by fungi in all evaluated years more frequently than those grown at Prague-Ruzyně (Tabs. 6, 7).

Neofabraea (9.77% of apples), *Penicillium* (2.05%), *Alternaria* (1.38%) and a sterile grey mycelium (1.34%) were the most frequently isolated taxa from apples grown in the integrated production system (Tab. 5). The apples grown in the integrated production system from orchards at Holovousy and Česká Skalice were damaged by postharvest diseases caused by fungi in 2013 and 2014 more frequently than those at Prague-Ruzyně. In 2015, the apples grown in orchards in the integrated production system at Holovousy and Česká Skalice were damaged by postharvest diseases caused by fungi less frequently than apples grown at Prague-Ruzyně (Tabs. 6, 7, 8).

There were also differences in the incidence of fungi causing postharvest disease between the investigated orchards and the years of harvest. The difference was recorded in the occurrence of the genus *Neofabraea* in both production systems between the orchard at Holovousy (on average 32.80% of apples from organic production and 11.37% of apples from integrated production) and the orchard at Prague-Ruzyně (2.13% of apples from organic production and 2.06% of apples

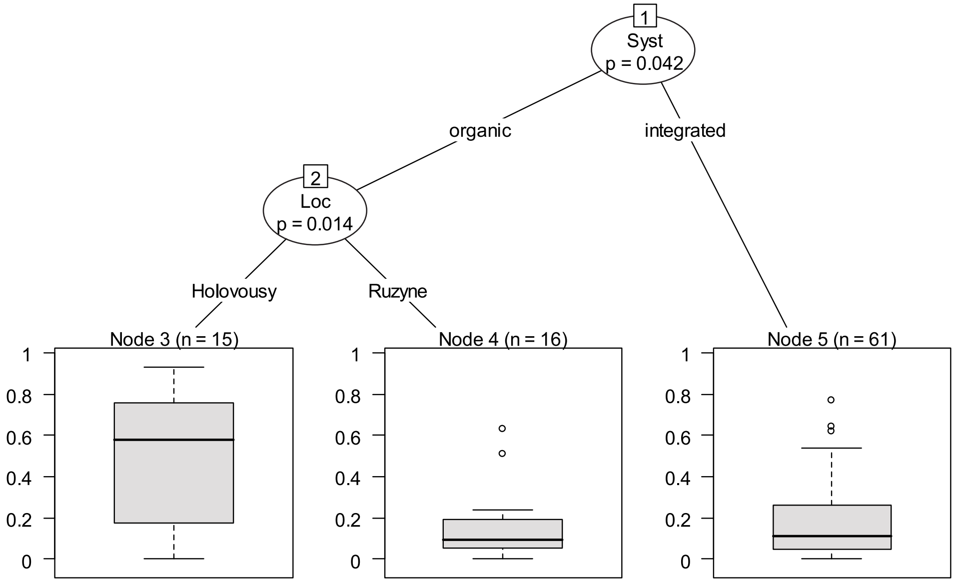

Fig. 3. Regression tree of factors (Loc – locality, Syst – production system) influencing infection intensity of sampled apple fruits (sample size n = 92). For each inner node, Bonferroni-adjusted P-values are given and infection intensity is displayed for each terminal node.

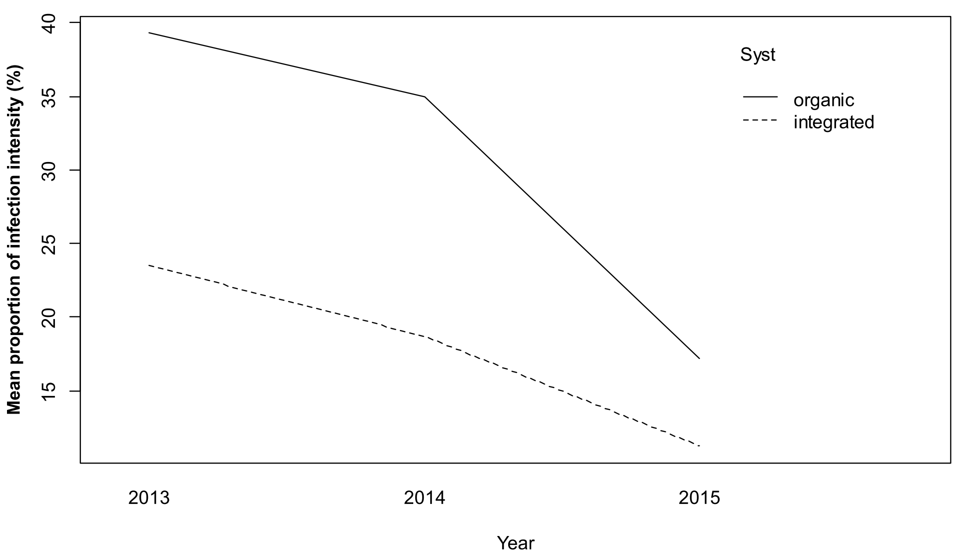

Fig. 4. Interaction plot for proportion of infection intensity in relation to plant production system (organic vs. integrated) in the years 2013–2015.

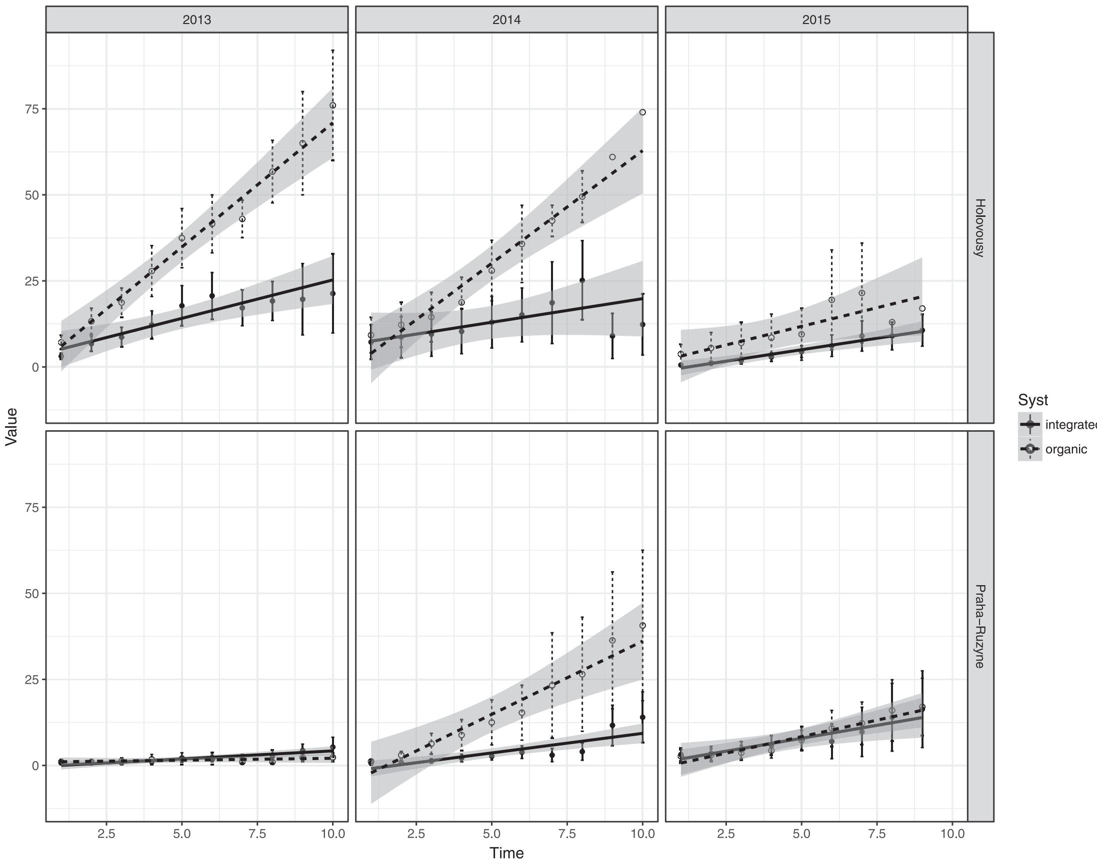

Fig. 5. Linear models with their 95% confidence bands (SE: grey) for intensity of fungal postharvest diseases of apple fruits from integrated (mean ± SE: full dots and unbroken line) and organic (mean ± SE: open dots and dotted line) production systems as a function of time from orchards at Holovousy and Prague-Ruzyně in the years 2013–2015.

from integrated production) (Tabs. 6, 7). The frequency of the genus *Neofabraea* in the orchard at Česká Skalice (14.22% of apples) was similar to the frequency in the integrated production system in the orchard at Holovousy (Tabs. 6, 7, 8).

Postharvest diseases of apples from particular orchards

A total of 4,200 apples from the orchard at Holovousy (1,500 organic apples and 2,700 apples grown in the integrated production system) were evaluated. 29.14% of them were damaged with postharvest diseases caused by fungi. In all evaluated years, apples grown in the organic production system were damaged more frequently than apples grown in the integrated production system. A total

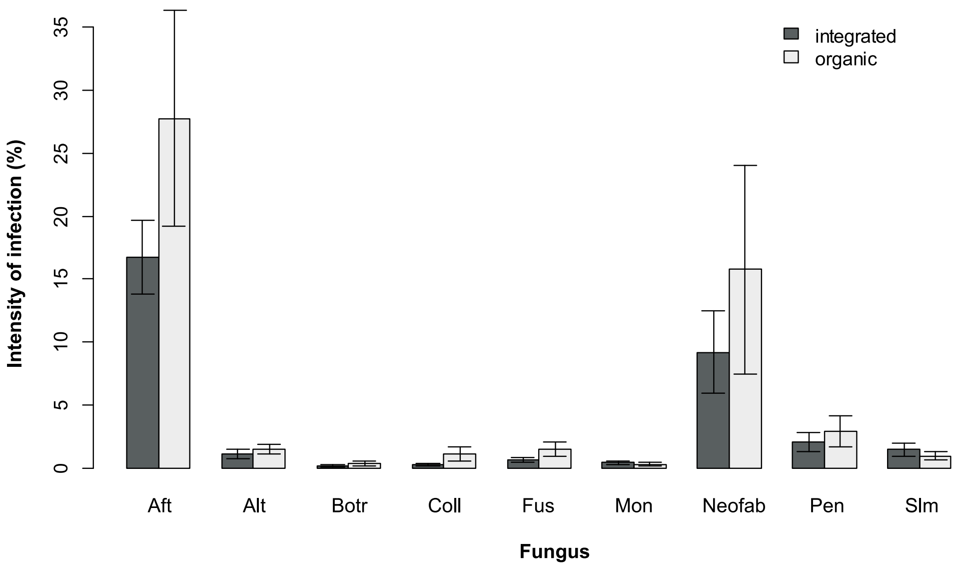

Fig. 6. Frequency of occurrence (mean ± SE) of the most important fungal taxa causing postharvest diseases in organic and integrated production systems.

Abbreviations: Aft – all fungal taxa, Alt – *Alternaria*, Botr – *Botrytis*, Coll – *Colletotrichum*, Fus – *Fusarium*, Mon – *Monilinia*, Neofab – *Neofabraea*, Pen – *Penicillium*, Slm – sterile light mycelium.

of 1,224 fungal isolates belonging to 18 taxonomic groups were obtained from this orchard. Differences between trees from integrated and organic production, cultivars and years were found. On average 44.33% and 20.70% of apples grown in the organic production system and those from the integrated production system, respectively, were damaged by fungi. Differences in fruit damage rates caused by fungi were observed between years of fruit evaluation. These rates were the highest in 2013 and the lowest in 2015 (Tab. 6).

The cultivars Topaz, Rosana and Golden Delicious grown in the organic production system were the most frequently damaged. Fruits of cultivar Florina grown in the integrated production system were the least frequently injured. Apples were the most frequently damaged by fungi in 2013 (59.14% of apples from organic production, 28.22% from integrated production) and the least frequently in 2015 (16.25% and 10.00%). The genera *Neofabraea* (32.80%), *Penicillium* and *Fusarium* (2.40%) were the most frequently recorded taxa on apples from organic orchards. *Neofabraea* (11.37%) and *Alternaria* (2.37%) were the most frequently isolated genera from apples grown in integrated production. Differences in occurrence of the most frequent fungal taxa between 2015 and both previous years (2013, 2014) were found. In 2013 and 2014, *Neofabraea* was the most frequently isolated fungal genus from apples grown in both cultivation systems, but in 2015 *Penicillium* was the most frequently isolated fungal genus in both cultivation systems. A total of 16 and 14 fungal taxonomic groups were isolated from apples grown in the organic integrated production system, respectively (Tab. 6).

At Prague-Ruzyně 3,200 apples (1,600 organic apples and 1,600 apples grown in the integrated production system) were evaluated. In 14.59% of them postharvest damage was observed. Differences between trees in the integrated and organic production systems, cultivars and years were found. In 2014, apples from trees grown in the organic production system were damaged more frequently than apples grown in the integrated production system, but in 2013 and 2015 apples from trees grown in the organic production system were damaged less frequently than apples grown in the integrated production system (Tab. 7).

A total of 467 fungal isolates belonging to 20 taxonomic groups were obtained from this orchard. On average 16.50% and 12.69% of apples grown in the organic and the integrated production systems, respectively, were damaged by fungi. Differences in fruit damage rates caused by fungi were observed between the years. These rates were the highest in 2014 and the lowest in 2013. The cultivar Melrose was the most frequently damaged in both the integrated (30.67% of apples) and organic production system (26.33%). Fruits of cultivar Rosana grown from organic production (5.67%) and Topaz (7%) and Šampion (7.5%) from integrated production were the least frequently injured (Tab. 4).

The apples were damaged most frequently in 2014 (19.17% of apples) and the least frequently in 2013 (5.25%). A sterile grey mycelium (3.44%), *Penicillium* (3.31%) and *Neofabraea* (2.13%) were the most frequently recorded taxa from organic apples. *Penicillium* (3.19%), a sterile grey mycelium (2.13%) and *Neofabraea* (2.06%) were the most frequently isolated genera from the integrated production. Differences in occurrence of the most frequent fungal taxa were observed between years. In 2014, the genus *Neofabraea* occurred more than twice as frequently than in 2013 and 2015 in both production systems. In 2015, the occurrence of the genus *Penicillium* was many times higher than in 2013 and 2014 in both production systems. A total of 16 and 18 fungal taxonomic groups were isolated from apples grown in the organic and the integrated production system, respectively (Tab. 7).

A number of 1,800 apples from the orchard at Česká Skalice were evaluated (all variants from integrated production). 17.56% of them were damaged by postharvest diseases caused by fungi. Topaz was the most frequently damaged cultivar (more than 30% of apples). Fruits of the cultivar Bohemia were the least frequently damaged (7.33%). A total of 316 fungal isolates from this orchard, belonging to 11 taxonomic groups were obtained. Differences between years were recorded. *Neofabraea* (14.22% of apples) and *Penicillium* (1.00%) were the most frequently found fungal taxa (Tab. 8).

Tab. 4. Incidence (%) of apples with symptoms of fungal postharvest diseases in 2013–2015.

Locality	Cultivar (variant)	Production system	Year			
			2013	2014	2015	Average
Česká Skalice	Bohemia	integrated	4	10	8	7.33
Česká Skalice	Goldstar	integrated	6	27	7	13.33
Česká Skalice	Šampion (A)	integrated	8	15	0	7.67
Česká Skalice	Šampion (B)	integrated	12	11	3	8.67
Česká Skalice	Topaz (A)	integrated	77	9	5	30.33
Česká Skalice	Topaz (B)	integrated	64	40	13	39.00
Holovousy	Angold	organic	36	44	40	40.00
Holovousy	Angold	integrated	26	80	36	47.33
Holovousy	Bohemia	integrated	54	25	8	29.00
Holovousy	Florina	organic	11	7	2	6.67
Holovousy	Florina	integrated	5	0	0	1.67
Holovousy	Golden Delicious	organic	74	–	–	74.00
Holovousy	Golden Delicious (A)	integrated	27	36	3	22.00
Holovousy	Golden Delicious (B)	integrated	19	18	2	13.00
Holovousy	Idared	integrated	16	6	12	11.33
Holovousy	Melrose	organic	60	–	–	60.00
Holovousy	Melrose	integrated	44	3	3	16.67
Holovousy	Rosana	organic	78	58	0	45.33
Holovousy	Rubín	organic	62	–	–	62.00
Holovousy	Rubín	integrated	54	16	9	26.33
Holovousy	Topaz	organic	93	77	24	64.67
Holovousy	Topaz	integrated	7	31	20	19.33
Prague-Ruzyně	Goldstar	organic	0	93	6	33.00
Prague-Ruzyně	Goldstar	integrated	13	18	3	11.33
Prague-Ruzyně	Melrose	organic	4	24	51	26.33
Prague-Ruzyně	Melrose	integrated	2	28	62	30.67
Prague-Ruzyně	Rosana	organic	8	6	3	5.67
Prague-Ruzyně	Rosana	integrated	4	16	7	9.00
Prague-Ruzyně	Rubín	organic	–	12	19	15.50
Prague-Ruzyně	Rubín	integrated	–	9	11	10.00
Prague-Ruzyně	Šampion	organic	–	8	11	9.50
Prague-Ruzyně	Šampion	integrated	–	1	14	7.50
Prague-Ruzyně	Topaz	organic	6	15	19	13.33
Prague-Ruzyně	Topaz	integrated	5	0	16	7.00

– = incidence not evaluated due to the small yield

Tab. 5. Incidence of fungal taxa isolated from apples with symptoms of postharvest diseases in 2013–2015 (% of all evaluated apples).
Abbreviations: org – organic production, int – integrated production. In the last columns, average values are given for each fungal taxon.
In the last three rows, absolute numbers of processed material and obtained results are given.

Fungal taxon	2013		2014		2015		Average	
	org	int	org	int	org	int	org	int
Acremonium spp.	0.00	0.00	0.00	0.05	0.00	0.05	0.00	0.03
Alternaria spp.	2.00	1.42	1.50	0.76	1.40	1.95	1.65	1.38
Aureobasidium pullulans	0.27	0.16	0.10	0.10	0.40	0.38	0.26	0.21
Botrytis spp.	0.09	0.21	0.80	0.00	0.20	0.24	0.35	0.15
Cladosporium spp.	0.09	0.42	0.30	0.05	1.00	0.48	0.45	0.31
Colletotrichum spp.	2.18	0.32	1.60	0.43	0.00	0.00	1.29	0.25
Epiccocum nigrum	0.09	0.11	0.00	0.00	0.00	0.05	0.03	0.05
Fusarium spp.	2.64	0.53	1.70	1.14	0.70	0.33	1.71	0.67
Monilinia spp.	0.00	0.00	0.40	0.67	0.40	0.14	0.26	0.28
Mucor spp.	0.00	0.05	0.10	0.00	0.00	0.00	0.03	0.02
Neofabraea spp.	30.00	18.11	16.90	10.95	2.70	1.05	16.97	9.77
Penicillium spp.	0.64	1.16	1.60	0.38	6.60	4.52	2.87	2.05
Phoma spp.	0.27	0.05	0.00	0.00	0.20	0.48	0.16	0.18
Phomopsis spp.	0.00	0.05	0.20	0.05	0.00	0.00	0.06	0.03
Sterile brown mycelium	0.00	0.00	0.00	0.00	0.20	0.10	0.06	0.03
Sterile grey mycelium	0.27	0.26	5.60	2.90	0.80	0.76	2.16	1.34
Sterile light mycelium	0.73	0.79	3.50	1.48	0.00	0.00	1.39	0.75
Sterile white-grey mycelium	0.00	0.00	0.00	0.00	0.60	0.38	0.19	0.13
Trichoderma spp.	0.00	0.00	0.00	0.00	0.10	0.05	0.03	0.02
Verticillium spp.	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.02
Venturia inaequalis	0.00	0.00	0.10	0.00	0.00	0.00	0.03	0.00
All fungal taxa	39.27	23.63	34.40	19.00	15.30	10.95	29.97	17.67
Total number of evaluated apples	**1100**	**1900**	**1000**	**2100**	**1000**	**2100**	**3100**	**6100**
Number of obtained fungal isolates	**432**	**449**	**344**	**399**	**153**	**230**	**929**	**1078**
Number of recorded taxa	**13**	**14**	**15**	**14**	**13**	**15**	**19**	**20**

Tab. 6. Incidence of the most important fungal taxa isolated from postharvest symptoms recorded on stored apples from the orchard at Holovousy (% of all evaluated apples).
Abbreviations: org – organic production, int – integrated production. In the last columns, average values are given for each fungal taxon.
In the last three rows, absolute numbers of processed material and obtained results are given.

Fungal taxon	2013		2014		2015		Average	
	org	int	org	int	org	int	org	int
Alternaria spp.	2.86	2.78	1.00	1.22	1.00	3.11	1.87	2.37
Botrytis spp.	0.00	0.22	0.25	0.00	0.00	0.11	0.07	0.11
Colletotrichum spp.	3.43	0.56	1.50	0.78	0.00	0.00	2.00	0.44
Fusarium spp.	3.86	0.89	2.25	2.00	0.00	0.00	2.40	0.96
Monilinia spp.	0.00	0.00	0.75	1.00	0.00	0.00	0.20	0.33
Neofabraea spp.	46.29	19.44	37.00	13.56	5.00	1.11	32.80	11.37
Penicillium spp.	0.86	1.67	0.75	0.67	6.75	3.89	2.40	2.07
Sterile light mycelium	0.71	1.22	1.50	1.33	0.00	0.00	0.73	0.85
Sterile grey mycelium	0.43	0.44	1.25	3.11	1.00	0.78	0.80	1.44
Other taxa	0.70	1.00	0.25	0.22	2.50	1.00	1.06	0.72
All fungal taxa	59.14	28.22	46.50	23.89	16.25	10.00	44.33	20.70
Number of stored apples	**700**	**900**	**400**	**900**	**400**	**900**	**1500**	**2700**
Number of fungal isolates	**414**	**254**	**186**	**215**	**65**	**90**	**665**	**559**
Number of recorded fungal taxa	**10**	**12**	**10**	**10**	**8**	**10**	**16**	**14**

Tab. 7. Incidence of the most important fungal taxa isolated from postharvest symptoms recorded on stored apples from the orchard at Prague-Ruzyně (% of all evaluated apples).
Abbreviations: org – organic production, int – integrated production. In the last columns, average values are given for each fungal taxon.
In the last three rows, absolute numbers of processed material and obtained results are given.

Fungal taxon	2013		2014		2015		Average	
	org	int	org	int	org	int	org	int
Alternaria spp.	0.50	0.25	1.83	0.67	1.67	1.67	1.44	0.94
Botrytis spp.	0.25	0.25	1.17	0.00	0.33	0.67	0.63	0.31
Colletotrichum spp.	0.00	0.00	1.67	0.00	0.00	0.00	0.63	0.00
Fusarium spp.	0.50	0.50	1.33	0.83	1.17	1.17	1.06	0.88
Monilinia spp.	0.00	0.00	0.17	0.83	0.67	0.50	0.31	0.50
Neofabraea spp.	1.50	1.50	3.50	3.17	1.17	1.33	2.13	2.06
Penicillium spp.	0.25	1.75	2.17	0.17	6.50	7.17	3.31	3.19
Sterile light mycelium	0.75	0.75	4.83	1.33	0.00	0.00	2.00	0.69
Sterile grey mycelium	0.00	0.00	8.50	4.50	0.67	1.17	3.44	2.13
Other taxa	0.75	1.00	1.16	0.50	1.65	4.15	1.55	1.99
All fungal taxa	4.50	6.00	26.33	12.00	13.83	17.83	16.50	12.69
Number of stored apples	**400**	**400**	**600**	**600**	**600**	**600**	**1600**	**1600**
Number of fungal isolates	**18**	**24**	**158**	**72**	**88**	**107**	**264**	**203**
Number of recorded fungal taxa	**8**	**9**	**13**	**10**	**13**	**15**	**16**	**18**

Tab. 8. Incidence of the most important fungal taxa isolated from postharvest symptoms recorded on stored apples from the orchard at Česká Skalice (% of all evaluated apples).
All records are from integrated production. In the last column, average values are given for each fungal taxon.
In the last three rows, absolute numbers of processed material and obtained results are given.

Fungal taxon	**2013**	**2014**	**2015**	**Average**
Alternaria spp.	0.17	0.17	0.50	0.28
Botrytis spp.	0.17	0.00	0.00	0.00
Colletotrichum spp.	0.17	0.33	0.00	0.17
Fusarium spp.	0.00	0.17	0.00	0.06
Monilinia spp.	0.00	0.00	0.00	0.00
Neofabraea spp.	27.17	14.83	0.67	14.22
Penicillium spp.	0.00	0.17	2.83	1.00
Sterile light mycelium	0.17	1.83	0.00	0.67
Sterile grey mycelium	0.17	1.00	0.33	0.50
Other taxa	0.50	0.17	1.17	0.61
All fungal taxa	28.50	18.67	5.50	17.56
Number of stored apples	**600**	**600**	**600**	**1800**
Number of fungal isolates	**171**	**112**	**33**	**316**
Number of recorded fungal taxa	**7**	**8**	**7**	**11**

DISCUSSION

Comparison of different production systems

Blažek et al. (2006) compared frequencies of occurrence of bitter rot (*Neofabraea vagabunda* – formerly *N. alba* or *N. malicorticis*), grey mould (*Botrytis cinerea*), brown rot (*Monilinia fructigena*) and blue mould (*Penicillium expansum*) of 30 apple cultivars in an orchard without chemical protection (= organic production system) and in an orchard with chemical protection (= integrated production system or chemical production system). They found lower frequencies of occurrence of the fungal postharvest disease on apples from trees with chemical protection than from those without chemical protection. They observed postharvest diseases caused by fungi most frequently on apples of cultivars Golden Delicious, Jarka, Lord Lambourne, Jonagold, Primadela, Rubín, Rubinstep, Šampion and Topaz. DeEll & Prange (1993) investigated postharvest diseases of two apple cultivars grown in organic and in conventional production systems and found that organic apples were damaged more frequently than apples grown conventionally. Blue mould (*Penicillium expansum*), grey mould (*Botrytis cinerea*) and bull's eye (*Neofabraea perennans*) were recorded most frequently. Jönsson et al. (2009) investigated postharvest diseases (brown rot caused by *Monilinia fructigena* and bull's eye caused by *Neofabraea perennans*) of two apple cultivars grown in organic and in integrated production systems. They found that organically grown apples were damaged more frequently than apples grown in a conventional way.

In the present study, apples grown organically were generally damaged by fungi (29.97% of apples) more than apples grown in the integrated production system (17.67%). Symptoms of postharvest diseases were recorded most frequently on cultivars Golden Delicious, Melrose, Rosana, Rubín and Topaz, but there were great differences between the orchards. Postharvest diseases were found most frequently in the Holovousy orchard. The obtained results are influenced by a lack of some data on the occurrence of fungi on apples from the organic production system in 2014 and 2015 and both production systems in 2013 (see Tab. 4), but the differences in total results are not distinctive.

Fungi live predominantly in plants asymptomatically, but some of them cause changes in plant appearance (plant diseases). Camatti-Sartori et al. (2005) investigated endophytic mycobiota of leaves, flowers and fruits of healthy apple trees growing in orchards with conventional, integrated and organic production systems. Fungi were recorded most frequently in plants (including fruits) grown organically. *Colletotrichum* spp., *Xylaria* spp. and *Botryosphaeria* spp. were dominant taxa in healthy fruits. Granado et al. (2008) found higher frequencies of fungi in healthy apples grown organically than in integrated production systems. The most common fungi belonged to 'white' and 'pink' yeasts, *Aureobasidium pullulans*, *Cladosporium* spp., *Alternaria* spp. and sterile filamentous fungi.

In integrated production, a higher amount and number of fungicides and other pesticides are used than in organic production. It influences the frequency of occurrence of all fungi living in the orchard and the fungal succession in apple trees (including fruits). Fungal succession in apples probably starts anew after each fungicide application and therefore endophytic fungi and fungi causing postharvest diseases occur in organically produced apples more frequently than in apples from integrated production.

Blažek et al. (2006) recorded symptoms of bitter rot (*Neofabraea*) and grey mould (*Botrytis cinerea*; together 32.48% and 14.74% of apples without chemical protection and with chemical protection, respectively) more frequently than those of brown rot (*Monilinia fructigena*; 1.57% and 0.3%) and blue mould (*Penicillium*; 5.56% and 2.00%). Ivić et al. (2012) found the species *Monilinia fructigena* (0–7.03% of apples per year), *Penicillium expansum* (0.1–0.53%), *Botrytis cinerea* (0.2–0.53%) in apples of cultivar Idared (stored in a storage room without temperature regulation), but *Neofabraea* spp. were recorded rarely. Konstantinou et al. (2011) isolated most frequently *Penicillium expansum*, *Botrytis cinerea*, *Alternaria tenuissima* and *Mucor pyriformis* from stored apples in Greece. In the present study, the frequency of occurrence of the various taxa differed between orchards. *Neofabraea* was isolated from 16.97% of apples from organic orchards and from 9.77% of apples from orchards with integrated production. The frequency of occurrence of this genus was very different between the orchards. The genus *Botrytis* was recorded from 0.35% of organic apples and from 0.15% of apples from integrated production, *Monilinia* from 0.26% and 0.28%, and *Penicillium* from 2.87% and 2.05%, respectively. Fungi of the latter genus produce a large amount of conidia, which can be dispersed by air. *Penicillium* can therefore spread easily and quickly not only in orchards but during storage, too. The production system did not influence the occurrence of the latter two genera significantly.

Influence of seasonal precipitation

Postharvest diseases caused by fungi were recorded much less frequently in 2015 than in 2013 and 2014. The precipitation in the 2015 vegetation season was 1.5 to 2 times lower than in 2013 and 2014. The amount of precipitation in the vegetation season during the study was the highest in 2013. In July 2015, the amount of precipitation at Holovousy and Česká Skalice was less than 13.5 mm, at Prague-Ruzyně it was less than 31 mm. The amount of precipitation at all three localities in August 2015 was generally similar as in July of the previous years. In September 2015, the amount of precipitation at Holovousy and Prague-Ruzyně was less than 8.5 mm, at Česká Skalice it was less than 19 mm (for details, see Fig. 1).

At what time (in which month) is deficiency of precipitation the most important for frequent occurrence of fungi causing postharvest diseases? In September the apples are already ripe. Therefore, the low frequency of occurrence of fungi causing postharvest diseases was probably caused by the lower precipitation before August. The average temperature was the highest in 2015 and therefore water evaporation was higher in 2015 than in 2013 and 2014. The higher temperature increased the influence of precipitation deficiency on the frequency of occurrence of fungi of apples. The fungicide application in 2015 was below average in comparison with 2013 and 2014 in all investigated orchards. The influence of the applied fungicides on the frequency of occurrence of fungi in 2015 was high, because the deficiency of precipitation made the impact of fungicides more effective. Fungi living asymptomatically in apples before harvest (*Neofabraea*, *Alternaria*, *Fusarium*, *Colletotrichum*) did not have good living conditions and did not colonise the fruit as much as in 2013 and 2014. Fungi from the genus *Penicillium*, which are most frequently recorded in storage and live rarely asymptomatically in healthy plants, can colonise the apples easier after the harvest.

Dominant fungi causing postharvest diseases

In the majority of papers dealing with the occurrence of fungal postharvest diseases of apples the fungi are identified without being isolated in pure culture (only based on symptoms) (e.g. DeEll & Prange 1993, Blažek et al. 2006, Jönsson et al. 2009) or without identification on species level. In the present study, a number of 2,007 fungi were isolated from apples with symptoms of postharvest diseases and identified using morphological features on genus level only. In many cases, molecular genetics characteristics (sequencing of DNA) are necessary for species identification, the use of which could provide deeper insight into these issues.

In the present study, the fungal genus *Neofabraea* (causing bitter rot of apples) was recorded most frequently in apples with symptoms of postharvest diseases in the orchards at Holovousy and Česká Skalice, but it was less frequently isolated from apples from the orchard at Prague-Ruzyně. Blažek et al. (2006) recorded symptoms of bitter rot and grey mould frequently. They did not separate bitter rot and grey mould, making it thus impossible to compare with our results. They suggest that bitter rot is caused by *Neofabraea vagabunda* (formerly *Neofabraea alba*, *Pezicula alba* and *Gloeosporium album*). *Neofabraea* spp. were also recorded in stored apples by other researchers. For example, Grantina-Ievina (2015) observed *Neofabraea* species as the most frequent of all fungal taxa (0.3–50.88% of apples) and identified the isolates as *N. vagabunda* and *N. malicorticis*. Similarly, Valiuškaittė et al. (2006) and Juhnevica-Radenkova et al. (2016) frequently found *Neofabraea* spp. in apples, too. Michalecka et al. (2016) and Pešicová et al. (2017) found *Neofabraea vagabunda* to be the most common species of the genus *Neofabraea* occurring in apples, but they recorded *N. perennans* and *N. kienholzii*, too. They did not find any strains of *N. malicorticis*. Pešicová et al. (2017) recorded *Neofabraea vagabunda*, *N. perennans* and *N. kienholzii* in one orchard. Therefore, it is almost impossible to name all strains of *Neofabraea* isolated from one orchard as *N. vagabunda* without sequencing their DNA.

Penicillium spp. are frequently isolated from decaying apples as fungi causing postharvest disease (Valiuškaitė et al. 2006, Konstantinou et al. 2011, Ivić et al. 2012, Grantina-Ievina 2015, Juhnevica-Radenkova et al. 2016). Many researchers (Konstantinou et al. 2011, Ivić et al. 2012, Juhnevica-Radenkova et al. 2016) only mention *Penicillium expansum*, but other species of the genus *Penicillium* can probably cause postharvest disease of apples, too. Amiri & Bompeix (2005) isolated seven *Penicillium* species from decaying apples (*P. chrysogenum*, *P. commune*, *P. digitatum*, *P. expansum*, *P. rugulosum*, *P. solitum*, *P. verrucosum*). Grantina-Ievina (2015) recorded four *Penicillium* species in apples (*P. brevicompactum*, *P. echinulatum*, *P. expansum*, *P. solitum*). Sanderson & Spotts

(1995) identified seven *Penicillium* species in decaying apples and pears (*P. aurantiogriseum*, *P. chrysogenum*, *P. commune*, *P. digitatum*, *P. expansum*, *P. roquefortii*, *P. solitum*), *P. expansum* and *P. solitum* being dominant species. Alwakeel (2013) found three *Penicillium* species (*P. chrysogenum*, *P. steckii*, *P. chrysogenum*) and *Aspergillus oryzae* in decaying apples, but did not isolate other fungi. He obtained the apples from supermarkets and therefore could record only fungi which quickly damage the fruits. In the present study, fungi from the genus *Penicillium* were recorded most frequently in 2015, when the frequency of occurrence of most other fungi was lower than in 2013 and 2014. Many taxonomic studies of the genus *Penicillium* were published and much knowledge of the taxonomy of *Penicillium* was obtained during the past 15 years. Therefore, it is irresponsible to classify all *Penicillium* isolates from stored apples into the species *P. expansum* without sequencing their DNA. The isolates of *Penicillium* in the present study probably belong to more *Penicillium* species.

Other fungal genera causing postharvest disease of apples are investigated less than *Neofabraea* and *Penicillium* and therefore less knowledge concerning their diversity is available. Fungi of the genus *Alternaria* are also frequently recorded as fungi causing postharvest diseases of apples (Valiuškaitė et al. 2006, Konstantinou et al. 2011, Ivić et al. 2012, Grantina-Ievina 2015). Konstantinou et al. (2011) recorded *Alternaria tenuissima* on stored apples in Greece, while Grantina-Ievina (2015) isolated *A. alternata* from apples. Granado et al. (2008) recorded *Alternaria* spp. in healthy apples frequently. Some *Alternaria* species live asymptomatically (as endophytes) in many plants (herbs, trees) and some *Alternaria* species are known as phytopathogens causing diseases of various plants. In the present study, *Alternaria* was recorded mainly in the orchards at Holovousy and Prague-Ruzyně in approximately 2% of apples.

In the present study, fungi of the genus *Fusarium* were isolated quite frequently in the Holovousy orchard. Sever et al. (2012) isolated *Fusarium avenaceum* (most frequently), *F. pseudograminearum*, *F. semitectum*, *F. crookwellense* and *F. compactum* from apples. Grantina-Ievina (2015) recorded *Fusarium* species in 0–2.2% of apples of different cultivars, classifying the isolates as *Fusarium avenaceum* and *Fusarium* spp. Konstantinou et al. (2011) identified *F. avenaceum* and *F. proliferatum* in stored apples in Greece. Many similar species were described in the genus *Fusarium* but identification of these species is very difficult. Just as for *Alternaria*, the *Fusarium* isolates obtained in the present study probably belong to more than one species, but without sequencing their DNA it is very difficult to classify them at species level.

CONCLUSIONS

Fungi causing postharvest diseases were more frequently recorded in fruits from apple trees from organic than integrated production. The difference was statistically significant. *Neofabraea* spp., *Penicillium* spp., *Alternaria* spp., a sterile grey mycelium and *Fusarium* spp. were the most frequently recorded taxa in both production systems.

Differences in occurrence of the most frequent fungal taxa causing postharvest disease between years were found. In 2013 and 2014, *Neofabraea* was the most frequently isolated fungal genus from apples grown in both production systems, but in 2015 *Penicillium* was the most frequent in all orchards. This was probably caused by the low precipitation in the 2015 vegetation season.

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